Supporting Information

Abrahamsson et al. 10.1073/pnas.0900189106

SI Methods

Flow-Cytometric Analysis and Cell Sorting. Mononuclear fractions were extracted from peripheral blood or bone marrow following Ficoll density centrifugation according to standard methods (1). Samples were analyzed fresh or subsequent to rapid thawing of samples previously frozen in 90% FCS and 10% DMSO in liquid nitrogen. $CD34^+$ cells were enriched from mononuclear fractions with the aid of immunomagnetic beads ($CD34^+$ Progenitor Isolation Kit, Miltenyi Biotec) and then stained with a mixture of antibodies (CD2, 3, 4, 8, 10, 14, 19, 20, 56, +/− glycophorin A, +/− CD7, +/− CD11b) specific for terminally differentiated cells to identify the lineage-positive (Lin^+) fraction (1). Subsequently, cells were washed and stained with a mixture of antibodies specific for HSC including CD34-APC, CD38-biotin detected with Streptavidin-PE-Cy7 and CD90-PE or a myeloid progenitor antibody mixture consisting of CD34-APC, CD38 Biotin detected with Streptavidin-PE-Cy7, CD45RA-FITC, and CD123-PE as previously described (1). Cells were analyzed and FACS purified according to established methods (1).

Lentiviral Production and Transduction. The lentiviral vector pHR2 was made by replacement of eGFP in pHR'tripCMV-GFP-SIN by a multiple cloning site (2). The GFP-firefly luciferase fusion (GLF) gene was subcloned from pJW.GFP-yLuc (gift of Dr. M.H. Bachmann, Stanford University School of Medicine, Stanford, CA) into pHR2 to generate pHR2-GLF. Lentiviral particles were prepared as previously described (2). The titer was determined by infection of 293T cells with several dilutions of the virus (MOI approximately 100) and analysis of GFP^+ cells by FACS 48 h later. Equal numbers (10^3-4×10^5) of normal or CML HSC, progenitors, and Lin^+ cells were sorted with the aid of a FACS Aria directly into 100 microliters of myelocult media (Stemcell Technologies) supplemented with cytokines (R&D Systems) including SCF (50 ng/ml), TPO (10 ng/ml), Flt3 ligand (50 ng/ml), and IL-6 (10 ng/ml) and transferred to a 96-well U bottom plate containing 100 microliters of cytokine-supplemented myelocult media per well (1). Cells were incubated in a 7% CO_2, 37 °C humidified incubator for 48 h and transduction efficiency was assessed by fluorescence microscopy-mediated GFP detection. Before transplantation cells were centrifuged for 5 min at $300 \times g$ and resuspended in 10 microliters of myelocult media before transplantation. For GSK3β virus preparation, GSK3β was amplified by PCR from Plasmid (Open Biosystems Clone ID 3357620 Accession BC000251), normal peripheral blood cDNA or the GMP fraction of a BC CML sample harboring the misspliced GSK3β isoform using the following primers:

FW primer: ctagtctagaaaggtgattcgcgaagagag

Rev primer: tcccccgggactggtggtttttcctgtgc

PCR products were then digested with XbaI and ClaI and cloned into a lentiviral backbone vector harboring IRES GFP under the control of a CMV promoter.

Transplantation and Engraftment Analysis. Immunocompromised (RAG2−/−γ_c−/−) mice deficient in T, B, and NK cells were bred and maintained in a barrier facility in the Moores University of California San Diego Cancer Center vivarium. Neonatal RAG2−/−γ_c−/− mice were transplanted within 48 h of birth intrahepatically with equal numbers (10^3-4×10^5 cells/mouse) of HSC, progenitors, or Lin^+ cells in a 10 μl volume with the aid of a Hamilton syringe (3). In 8 experiments ($n = 30$ mice) involving normal marrow or cord blood and 12 experiments involving blast crisis CML ($n = 43$ mice), neonatal RAG2−/−γ_c−/− mice were transplanted with equivalent numbers (10^3 to 4×10^5) of luciferase-GFP transduced HSC, progenitors or Lin^+ cells. Bioluminescent imaging, used to monitor engraftment kinetics, started at week 3 posttransplant and continued weekly until the time of sacrifice. Briefly, this involved i.p. administration of 200 μl of luciferin (Caliper, Inc.) and bioluminescent imaging of isofluorane anesthetized mice with the aid of a highly sensitive in vivo imaging system (IVIS 200; Caliper, Inc.). Bioluminescent imaging of nontransplanted mice served as a negative control for each experiment. When moribund or at 8–12 weeks posttransplant, mice were euthanized with inhaled CO_2. Single cell suspensions of hematopoietic organs including blood, liver, spleen, marrow, and thymus as well as tumors were analyzed for the presence of human cells via FACS. Following ammonium citrate lysis of red blood cells for 10 min at 4 °C, an anti-human Fcγ receptor blocking antibody (Miltenyi, Human CD34 Progenitor Isolation kit) was added (dilution of 1/50) and anti-mouse Fcγ receptor (CD16/CD32, BD PharMingen) for 30 min. Cells were then stained with mouse monoclonal antibodies (Invitrogen) specific for human CD45 (CD45-APC), CD33 and CD14 (both conjugated to FITC), CD19 (CD19-PE-Cy7), and CD3 (CD3-PE, BD PharMingen). Hematopoietic organs from nontransplanted mice and normal human peripheral blood served as negative and positive controls, respectively. Mice with greater than 1% human cells were deemed to be engrafted. Engrafted human $CD45^+$ cells were sorted into RLT buffer (Qiagen RNeasy) and RNA extracted with the RNeasy kit as previously described for BCR-ABL analysis (1). In secondary transplantation experiments, human cells from marrow, spleen, liver, and tumors were sorted into 100 μl of myelocult media for intrahepatic transplantation into neonatal RAG2−/−γ_c−/− mice.

Nested P210 BCR-ABL RT-PCR. Total RNA was isolated using a RNeasy Mini Kit (Qiagen) (1) and quantified using a NanoDrop ND-1000 Spectrophotometer. In primary transplantation experiments, preparation of cDNA from 2 μg of total transplanted mouse tissue RNA was carried out using a "High Capacity cDNA Archive Kit" (ABI). Then, P210 amplification was performed with 5 μl cDNA and 21 μl of reaction mixture consisting of 1x Buffer II (ABI), 2.5 mM $MgCl_2$, 0.2 mM dNTP's, NBI^+ (4 ng/μl), $ABL3^-$ (4.2 ng/μl) and 5 U/reaction of AmpliTaq Gold (ABI). The second round of P210 BCR-ABL amplification was performed with 1 μl of amplified product and 20 μl of reaction mixture consisting of 1x Buffer II (ABI), 1.75 mM $MgCl_2$, 0.2 mM dNTP's, 4.4 ng/μl CA3-, 3.4 ng/μl B2A, and 5U/reaction of AmpliTaq Gold (ABI). In subsequent transplantation experiments, a one-step RT-PCR kit (Qiagen Inc.) was used with the BCR-ABL same primers. Electrophoresis of amplified products was performed on a 1.5% agarose gel with expected product sizes for b2a2 and b3a2 of 383bp and 458bp, respectively (1).

GSK3α, GSK3β, and β-catenin FACS Analysis. Normal and CML $CD34^+$ enriched cells from CP, AP, and BC CML peripheral blood or marrow were first stained with lineage antibodies and then stained with either hematopoietic stem cell or progenitor cocktails of antibodies followed by fixation with 0.8% paraformaldehyde for 10 min. Cells were then washed by centrifugation at 1,200 rpm for 5 min and resuspended in HBSS with a rabbit monoclonal antibody specific for the first 20 N-terminal amino acids of human GSK3β (#9315, Cell Signaling Technology)

diluted 1:50 in 0.15% Saponin (TCI America) and 10% goat serum (Sigma-Aldrich) for 45 min at 4 °C. Cells were then washed and stained with an Alexa 405-conjugated goat anti-rabbit antibody (Molecular Probes) diluted 1:400 for 45 min at 4 °C to detect the GSK3β expressing cells. Control stain with only the Alexa-405 goat anti-rabbit antibody was also done to evaluate the nonspecific background Rb staining. The isotype control for Rb-GSK3β antibody was Rb IgG (Jackson ImmunoResearch). Mean fluorescence intensity was analyzed with the aid of a FACS Aria and FlowJo software. FACS analysis of activated β-catenin expression was performed in a similar manner on patient samples and single cell suspensions of livers from mice transplanted with blast crisis CML $CD34^+CD38^-Lin^-$ or $CD34^+CD38^+Lin^-$ cells with the proviso that the β-catenin antibody (clone 8E7, Upstate) was directly labeled (Xenon kit, Invitrogen) with Alexa-405 according to the manufacturer's specification. Isotype control for β-catenin antibody was Ms IgG_1 (Chemicon International). FACS analysis of GSK3α (#9338, Cell Signaling Technology) protein expression was performed by directly labeling the antibody (Xenon kit, Invitrogen) with Alexa-405 according to the manufacturer's specifications. Isotype control for the Rb-GSK3α antibody was Rb IgG (Jackson ImmunoResearch).

Quantitative RT-PCR. Hematopoietic stem and progenitor (1,000 to 50,000) cells were sorted directly into RLT Buffer and total RNA was isolated using RNeasy Micro Kit (Qiagen), according to manufacturer's protocol. An SYBR Greener two-Step Q-RT-PCR Kit for the iCycler (Invitrogen) was then used to synthesize cDNA and assess GSK3β relative transcript quantities according to the manufacturer's protocol. Briefly, 8 μl of 4 to 75 ng/μl of RNA were mixed with RT Reaction Mix and RT Enzyme Mix and incubated at 25 °C for 10 min, followed by 50 °C for 30 min and finally 85 °C for 5 min. The tubes were then chilled and 1 μl of RNase H was added to the reaction followed by a 20 min incubation at 37 °C. The quantitative PCR (Q-PCR) reaction was performed in duplicate using 2 μl of the template in 25 μl reaction volume containing SYBR Greener Super Mix and 0.4 μM of each forward and reverse primer.

The following primers were used:
GSK3β Forward: gacatttcacctcaggagtgc
GSK3β Reverse: gttagtcgggcagttggtgt
c-myc Forward: acgtctccacacatcagcac
c-myc Reverse: cgcctcttgacattctcctc
Axin2 Forward: agcctaaaggtcgtgtgtgg
Axin2 Reverse: acaggatcgctcctcttgaa
HPRT Forward: cgtcttgctcgagatgtgatg
HPRT Reverse: tttatagccccccttgagcac

Relative values of transcripts were determined according to a standard curve. Values were then normalized to HPRT values (1).

Allele-Specific QPCR Assay. AQPCR assay was developed for the specific and quantitative detection of misspliced GSK3β and FL-GSK3β from 4 ng RNA.

The following primers were used:
mGSK3β (Ex7) Forward: ctgtgtgttggctgagctgt
mGSK3β (Ex + 10 junction) Reverse: gggtcggaagaccttgatta
FLGSK3β (Ex7–2) Forward: tttccaggggatagtggtgt
FLGSK3β (Ex8 + 9 junction) Reverse: cctgacgaatccttagtcca

Wnt Mutation Screening

Wnt/β-catenin Signaling Pathway Mutation Analysis. Targeted Wnt/β-catenin pathway regulator genomic DNA mutation and cDNA analysis was performed on normal or CML FACS-sorted $CD34^+CD38^-Lin^-$, $CD34^+CD38^+Lin^-$, HSC, GMP, and lineage-positive cells. Wnt/β-catenin pathway genes analyzed included β-catenin, GSK3β, adenomatous polyposis coli (APC), axin 1, c-myc, cyclin D1 (CCND1), and LEF-1.

Sample Preparation and RNA Extraction. Total RNA was extracted from snap-frozen cells and colonies using either the RNeasy Mini Protocol (Qiagen) or TRIZOL reagent (Invitrogen) according to the instructions of the manufacturers. All samples were quantified using the NanoDrop ND-1000 Spectrophotometer and resuspended at working concentrations of 100 ng/ul in RNase free water.

RT-PCR. First strand cDNA synthesis of 500 ng of purified RNA was performed with the SuperScript III First-Strand Synthesis System for RT-PCR with Platinum Taq (Invitrogen) and 50 ng of random hexamers according to the manufacturer's recommendations in individual tubes for each RNA sample with 200U of the SuperScript III RT enzyme. PCR was performed using 100 ng of the cDNA as template in separate 50 μl reactions which consisted of final concentrations of 1.25U of HotMaster TaqDNA Polymerase (Eppendorf), HotMaster Taq Buffer with 2.5 mM Mg2+ (25 mM Tris-HCL pH 8.0, 35 mM KCL, 0.1 mM EDTA, 1 mM DDT, 50% glycerol, 0.5% Tween20, 0.5%IGEPAL CA-630 and stabilizers), 2 mM of each dNTP, 0.2 μM of each sense and anti-sense primer. Reverse transcription and PCR cycling steps were carried out in a MJ Research Dyad thermocycler. The conditions for first strand cDNA synthesis were 65 °C for 5 min, followed by 25 °C for 10 min and 50 °C for 50 min. Reactions were terminated at 85 °C for 5 min. PCR amplification of the newly synthesized cDNA performed by 35 cycles of denaturation (94 °C, 15 sec), annealing (55 °C, 30 sec), and extension (68 °C, 2 min) followed by a final extension step of 1 cycle at 68 °C for 5 min. GSK3β outer PCR primers used in the PCR amplifications were (forward) 5′-aaggtgattcgcgaagagag -3′ and (reverse) 5′-actggtggtttttcctgtgc -3′ These primers amplify a 1343 bp cDNA product that contains the ORF (ORF) of GSK3β. Multiple nested inner primers were used to produce full double stranded sequencing coverage of the entire ORF. These nested inner primers are available upon request.

Sequence Scanning. Sequence analysis of the β-catenin, APC, axin 1, c-myc, LEF-1, cyclin D1, and GSK3β cDNA PCR products were conducted with SURVEYOR mismatch cleavage analysis using the WAVE-HS System (Transgenomic) to detect sequence anomalies such as mutations and unusual splice forms. Aliquots of PCR product (3–15 μl) were scanned for mutations by Surveyor mismatch cleavage and identified with bidirectional sequence analysis on an ABI 3100 sequencer using BigDye V3.1 terminator chemistry (Applied Biosystems, Inc.). For semiquantitative determination of mutant and normal allele frequencies, relative peak areas of Surveyor mismatch cleavage products were determined after normalization and comparison to reference controls using the WAVE Navigator software.

DNA sequencing and Analysis

Primers. GSK3β ex5 For- tgagtgccagtaatccatagca
GSK3β ex5 Rev - cacagctgcactagtttatacttcttg
GSK3β ex6 For- tttttgcagagcaaggtgat
GSK3β ex6 Rev- aagcagtgcctggaaagtataa
GSK3β ex7 For- tggtctggtttcctctttgg
GSK3β ex7 Rev- gaagcaatccaaggtcacgt
GSK3β ex8 For- catgagctttgggggacata
GSK3β ex8 Rev- agtacactgccccttaccttgc
GSK3β ex9 For- ccccacaccatggatttct
GSK3β ex9 Rev- gaatcacccaagaggctgac
GSK3β ex10 For- gaaggtcctttcccaaatgc
GSK3β ex10 Rev- tgtccgtttttgtcctccac
GSK3β ex11 For- gattacaggcgtgagccact

GSK3β ex11 Rev- cttgttggtcaggctggtct

PCR products were obtained using the above-mentioned primers, amplifying the corresponding exon and splice junctions. Excess PCR primers were removed from 10 μl of PCR product using the Ampure PCR Purification system (Agencourt). Purified product was eluted in 30 μl of deionized water. Standard reaction chemistry using BigDye v. 3.1 and cycle sequencing were used as per the manufacturer's recommendations. Cycle sequencing products were purified using CleanSeq reagents (Agencourt). Purified sequencing products were eluted in 40 μl of 0.01 μM EDTA and 30 μl was run on an ABI 3100 Genetic Analyzer. DNA sequence was analyzed using ABI Sequence Analysis v3.7, Sequencher (GeneCodes), and Mutation Surveyor (Softgenetics). The reference *GSK3*β (NM_002093) sequence was downloaded from the University of California San Cruz Genome Bioinformatics webpage using the March 2006 assembly.

Lentiviral GSK3β Transduction Experiments. GSK3β was amplified by PCR from Plasmid (Open Biosystems Clone ID 3357620 Accession BC000251) or peripheral blood cDNA from a normal donor or the GMP fraction of a BC CML patient using the following primers:

Forward primer: ctagtctagaaaggtgattcgcgaagagag

Reverse primer: tccccgggactggtggtttttcctgtgc

PCR products were then digested with XbaI and ClaI and cloned into a lentivirus backbone harboring IRES GFP under the control of a CMV promoter for in vitro and in vivo assays. For these assays, CP CML or BC CML peripheral blood progenitor cells were FACS-ARIA sorted into 96-well plates containing myelocult media (Stemcell Technologies) supplemented with Flt3 ligand (3 μg/ml), Interleukin-6 (1 μg/ml), stem cell factor (4 μg/ml), and thrombopoietin (0.625 μg/ml). Sorted CML progenitor cells (2–4 $\times$ 10^4 cells/well) were transduced with lentiviruses expressing full length GSK3β (FL-GSK) or GSK3β deleted in exon8 and 9 (*m*-GSK) as well as lentiviral luciferase-GFP (GLF) to permit bioluminescent imaging of engraftment in transplanted mice. Cells were washed 48 h after transduction and transplanted intrahepatically into neonatal RAG2$-/-\gamma_c-/-$ mice (17). For in vitro assays, CD34-enriched cells from CML peripheral blood were plated (10^4-10^5 cells/well) in a 96-well plate with myelocult media (Stemcell Technologies) supplemented with Flt3 ligand (50 ng/mL), Interleukin-6 (10 ng/mL), stem cell factor (50 ng/mL), and thrombopoietin (10 ng/mL). Lentiviruses expressing FL-GSK3, misspliced GSK3, or GLF were then added to each well. Cells were harvested 7 days after transduction for β-catenin FACS analysis performed as described above. For transduction efficiencies, see Fig. S6

LEF/TCF Reporter Assay. Lentivirus expressing GFP from a LEF/TCF responsive element promoter was a kind gift from Dr. Karl Willert at University of California San Diego, La Jolla, CA. GMP from normal cord blood, BC CML, and 2° BC CML tumors were FACSAria sorted into 96-well plates containing myelocult media (Stemcell Technologies) supplemented with Flt3 ligand (50 ng/mL), Interleukin-6 (10 ng/mL), stem cell factor (50 ng/mL) and thrombopoietin (10 ng/mL). Sorted GMP (700–5,000 cells/well) were transduced with a lentiviral LEF/TCF GFP reporter (1). Cells were cultured for 6–7 days before analyzing the samples on a FACSCaliber for GFP expression.

1. Jamieson CH, et al. (2004) Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. *N Engl J Med* 351:657–667.
2. Breckpot K, et al. (2003) Lentivirally transduced dendritic cells as a tool for cancer immunotherapy. *J Gene Med* 5:654–667.
3. Traggiai E, et al. (2004) Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* 304:104–107.

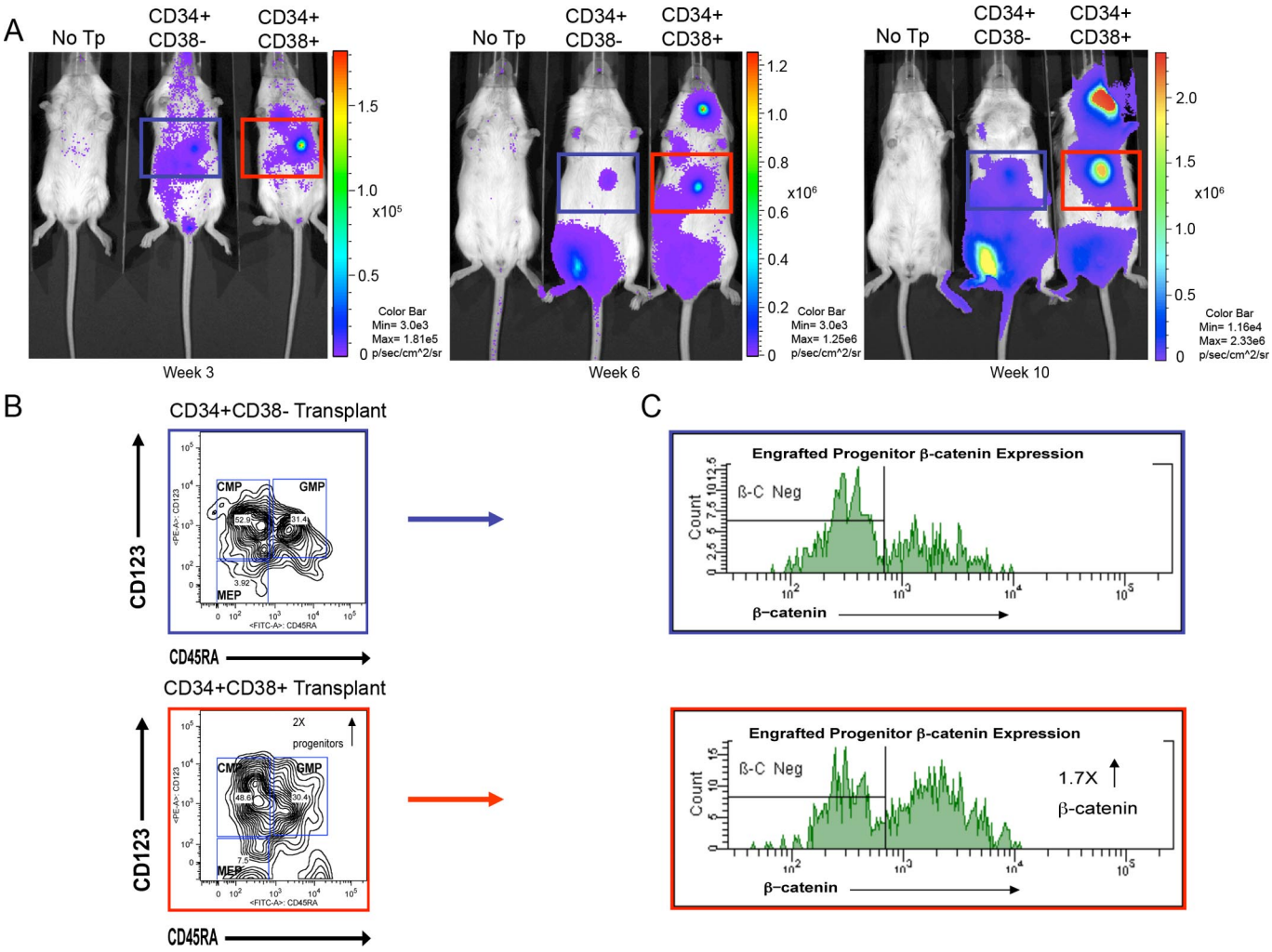

Fig. S1. Elevated β-catenin expression and engraftment of human BC CML progenitors analysis. (*A*) Bioluminescence imaging (IVIS 200, Caliper, Inc.) on weeks 3, 6, and 10 following intrahepatic transplantation of neonatal RAG2$-/-\gamma_c-/-$ mice (n = 3 experiments) revealed enhanced hepatic engraftment of BC CML 34^+38^+Lin$^-$ progenitor (1–4 × 10^5) cells (red square) compared with BC CML $CD34^+CD38^-Lin^-$ (1–4 × 10^5) cells (blue square). Untransplanted mice served as a negative control (3). (*B*) FACS analysis revealed a 2-fold increase (standard deviation = 0.09) in human myeloid progenitors (CMP, GMP, and MEP) hepatically engrafted in BC CML $CD34^+CD38^-Lin^-$ cell (red square) transplanted mice compared with those that received an equivalent number of BC CML $CD34^+CD38^-Lin^-$ (blue square) cells (n = 3 experiments). (*C*) FACS analysis also revealed a 1.7-fold (SD = 0.07) elevation in activated β-catenin in progenitors derived from transplanted BC CML $CD34^+CD38^+$ cells compared with blast crisis $CD34^+CD38^-$ cells.

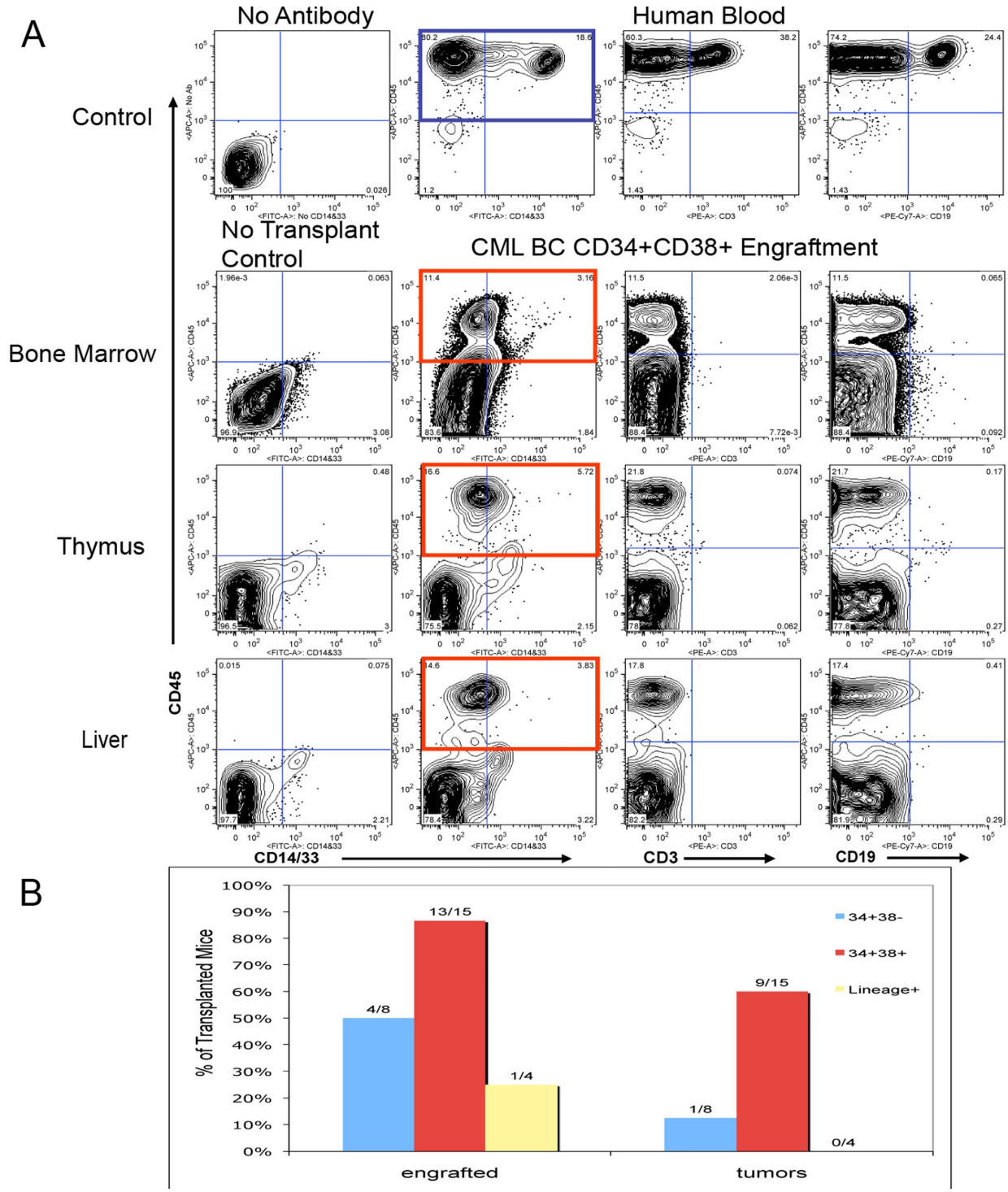

Fig. S2. Enhanced leukemic engraftment of misspliced GSK3β expressing BC CML progenitors. (*A*) FACS analysis ($n = 3$ experiments) of human engraftment in hematopoietic organs including bone marrow, thymus, and liver of RAG2$-/-\gamma_c-/-$ mice transplanted with (4×10^4-4×10^5) BC CML progenitor cells. Analysis shows predominant human myeloid (CD45, CD14, and CD33) engraftment in all hematopoietic organs analyzed (red box). Normal human blood CD45, CD14 and CD33 expression (blue box) served as a positive control. (*B*) There was increased human leukemic engraftment including granulocytic sarcoma (tumor) formation in RAG2$-/-\gamma_c-/-$ mice transplanted with BC CML CD34$^+$CD38$^+$ cells compared with mice transplanted with BC CD34$^+$CD38$^-$ or Lineage$^+$ cells as detected by bioluminescence imaging and confirmed by FACS analysis of single cell suspensions and/or BCR-ABL RT-PCR analysis.

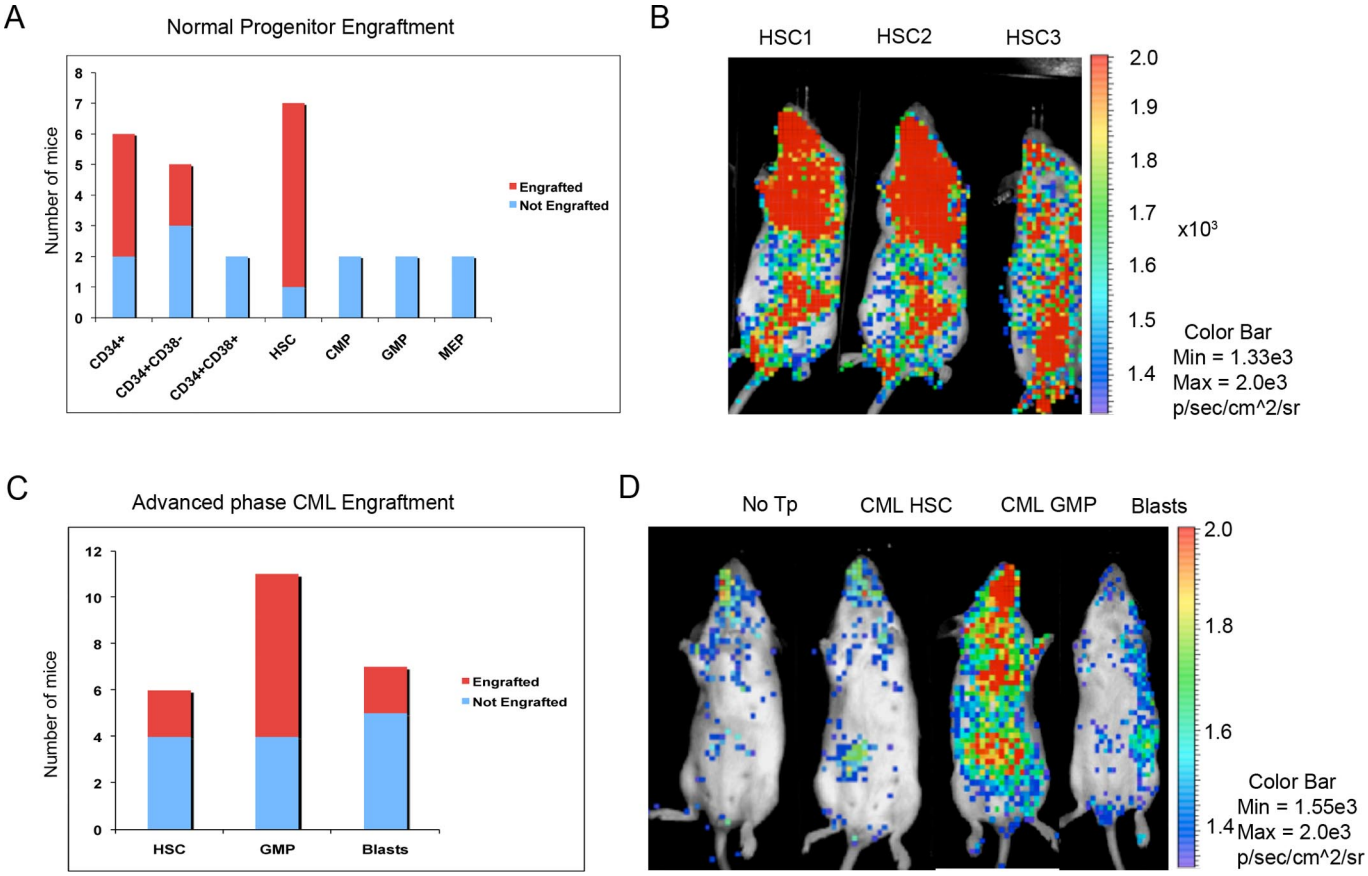

Fig. S3. Increased in vivo self-renewal capacity of myeloid Blast Crisis GMP. (*A*). In 7 experiments, 26 neonatal RAG2−/−γ_c−/− mice were transplanted with untransduced normal cord blood or marrow derived $CD34^+Lin^-$, $CD34^+CD38^-Lin^-$, $CD34^+CD38^+Lin^-$, HSC, CMP, GMP, or MEP. Human engraftment (≥1% human CD45 expression) was analyzed between 8 and 12 weeks posttransplantion by FACS in liver, bone marrow, thymus, and spleen. Normal HSC gave rise to the highest levels of engraftment (red; engrafted) while committed myeloid progenitors did not engraft long term (blue; not engrafted). (*B*) In 8 experiments, 30 neonatal RAG2−/−γ_c−/− mice were transplanted with normal human HSC or progenitor cells transduced with lentiviral GLF. Engraftment kinetics were monitored by weekly bioluminescence imaging (IVIS, Caliper, Inc.). These images demonstrate levels of bioluminescence produced 9 weeks postintrahepatic transplantation of mice with HSC derived from 3 separate normal marrow samples (HSC1, HSC2, HSC3). (*C*) In 6 experiments, 24 neonatal RAG2−/−γ_c−/− mice were transplanted with equivalent numbers of BC CML blood- or marrow-derived HSC, GMP, or blasts. Engraftment was analyzed as described above. BC GMP had the most robust long-term engraftment potential. (*D*) In 12 experiments, 43 neonatal RAG2−/−γ_c−/− mice were transplanted with advanced phase HSC, CMP, GMP, MEP, or blasts lentivirally transduced with luciferase, and engraftment kinetics were monitored by weekly bioluminescence imaging. Images demonstrate relative levels of bioluminescence at 9 weeks posttransplantion for HSC, GMP and blasts compared with no transplant (No Tp) controls.

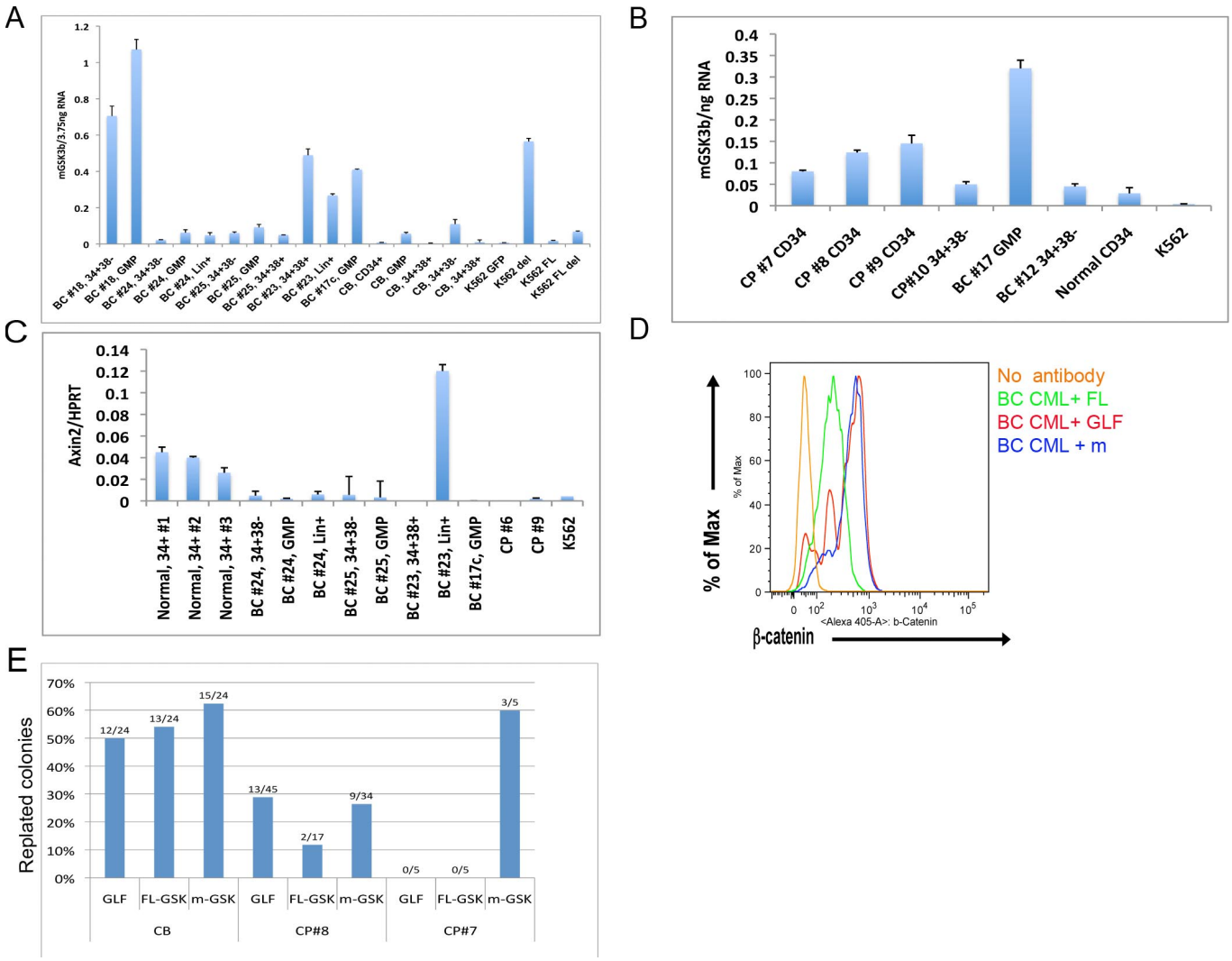

Fig. S4. Deregulation of Wnt pathway mediators in CML. (*A*) Isoform specific Q-PCR confirmed increased levels of misspliced GSK3β (m-GSK) transcripts in myeloid BC CML GMPs compared with normal CD34$^+$ progenitor cells. K562 cells transduced with GFP of different splice isoforms served as controls. (*B*) *m*-GSK3β isoform specific Q-PCR also revealed increased *m*-GSK3β transcripts in 3 additional chronic phase CML progenitor samples (#7, #8, #9). (*C*) Q-PCR revealed low Axin2 expression levels (normalized to HPRT) in all myeloid BC CML #17 fractions (HSC, GMP, CMP, and Lin+) as well as CP CML progenitors from CP samples #6 and #9 compared with 3 normal CD34 enriched samples. Axin 2 transcript levels were also reduced in K562, a myeloid BC CML cell line, underscoring the context specificity of Wnt signaling pathway deregulation. (*D*) FACS analysis employing an antibody specific for activated β-catenin revealed higher levels of activated β-catenin in control GLF-transduced (BC CML + GLF; red) and misspliced GSK3β-transduced (BC CML + m; blue) tumor-derived progenitors compared with full-length GSK3β (BC CML + FL; green) transduced progenitors. Tumor cells stained with all progenitor antibodies but no activated β-catenin antibody served as a negative control (orange). (*E*) Colony replating assays performed with FACS-sorted GFP$^+$ normal and CP CML progenitors demonstrated increased replating potential of lentiviral misspliced GSK3β-GFP (*m*-GSK) transduced CP progenitors compared with full-length GSK3β–GFP (FL-GSK) or luciferase-GFP (GLF) transduced controls. Cord blood (CB) progenitor replating was unaffected further emphasizing the cell type and context specificity of misspliced GSK3β on β-catenin activation and self-renewal capacity.

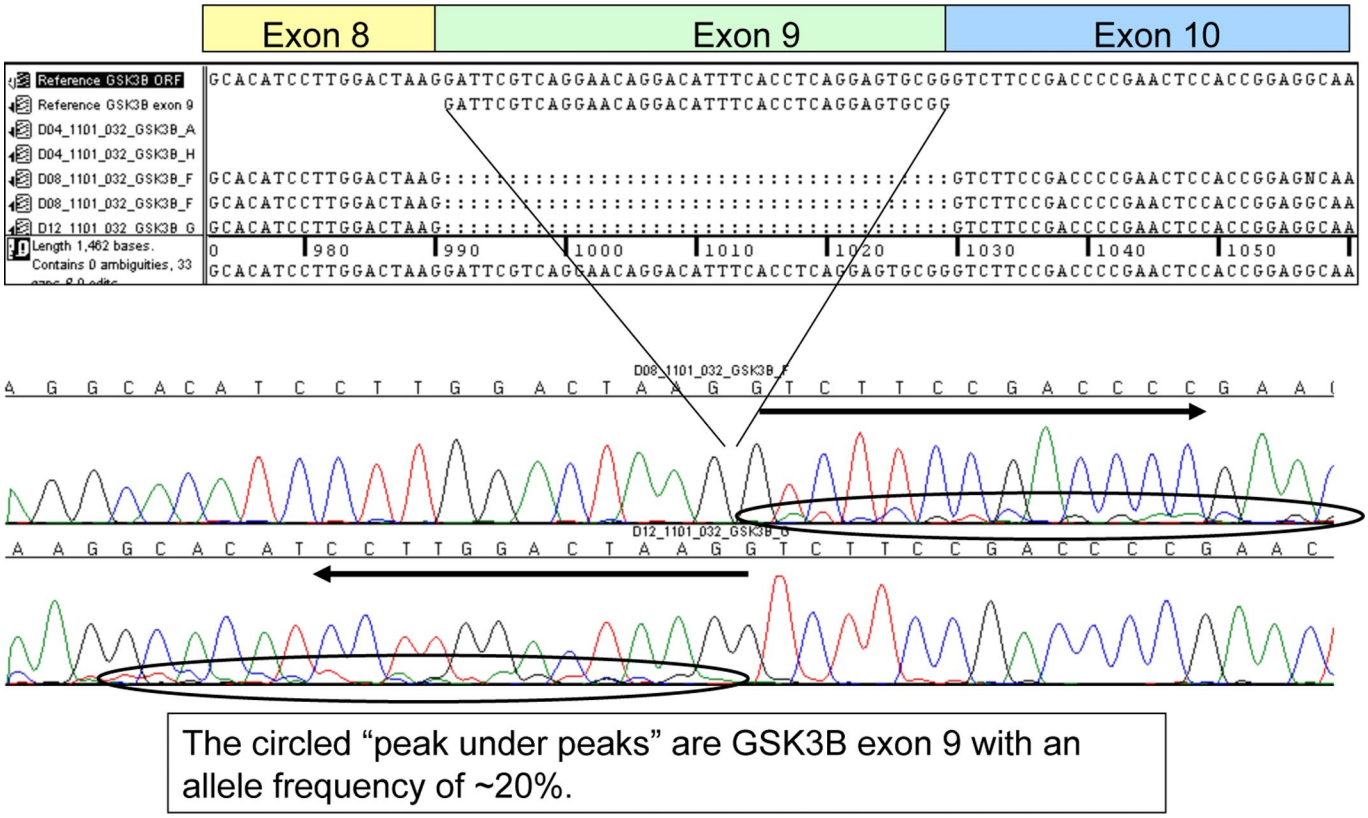

Fig. S5. GSK3β cDNA sequencing analysis of normal progenitors. HSC, GMP, CD34-enriched, and Lin$^+$ cells from normal marrow ($n = 2$), cord blood ($n = 2$), and peripheral blood ($n = 5$) samples were subjected to GSK3β cDNA sequencing. This analysis showed a deletion of GSK3β exon 9. Double-stranded sequencing of this region revealed that exon 9 is abolished in 20% of transcripts and represents an alternative splice isoform of GSK3β.

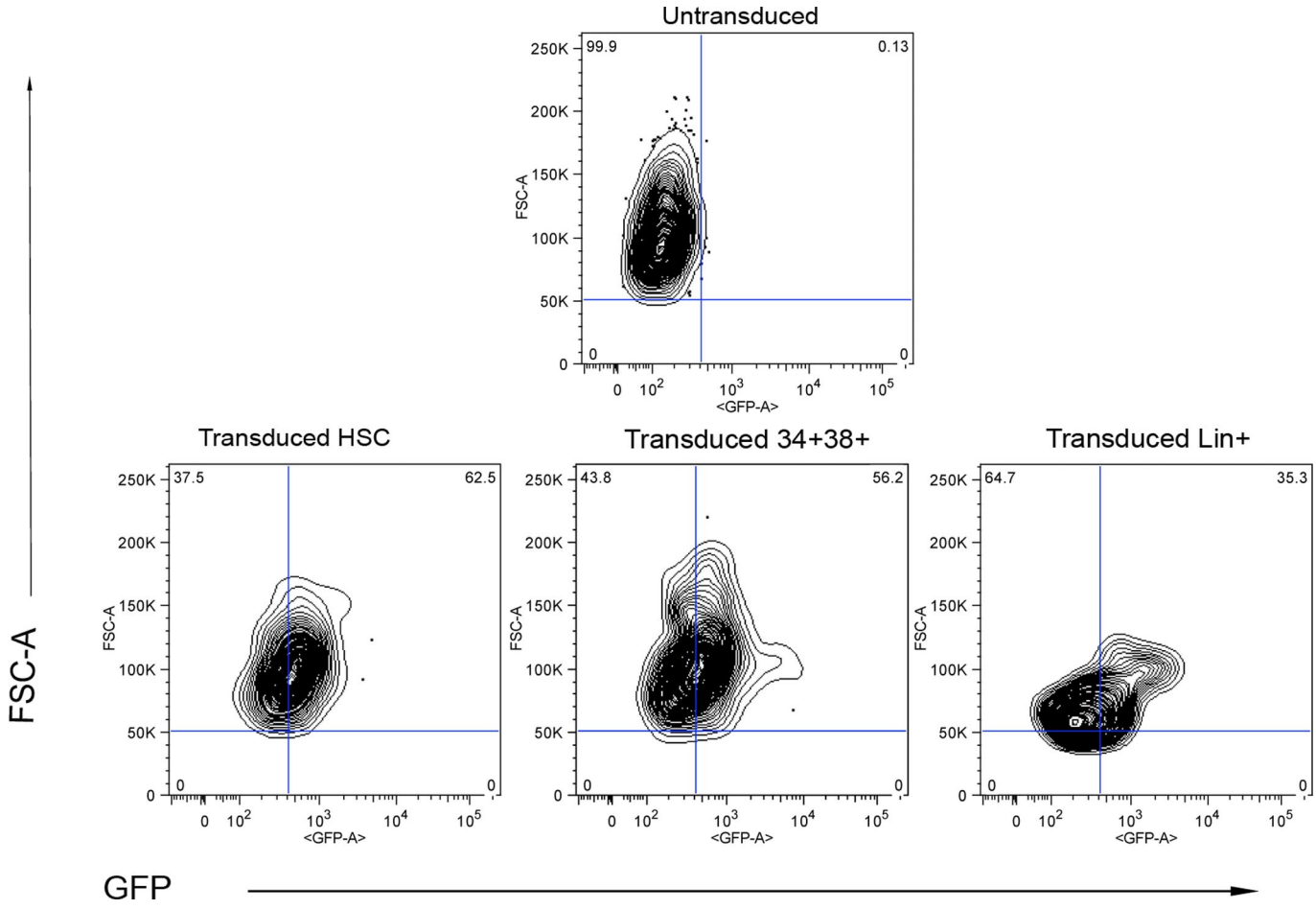

Fig. S6. Lentivirally enforced GSK3β expression. Lentiviral GSK-GFP transduction efficiency was assessed by FACS detection of GFP expression in transduced cells and found to be equivalent between normal cord blood HSC and progenitors ($34^{+}38^{+}Lin^{-}$) while $Lineage^{+}$ populations had slightly lower levels of GFP-expression.

Table S1. Patient Characteristics

Patient number	Sex/Age	Date	Phase of CML	WBC count, K/mm^3	% PB, Blasts	Treatment at time of sample acquisition
01	F/72	3/8/05	Chronic phase	17.8	0	Imatinib 400 mg daily
02*	F/59	4/6/05	Chronic phase	88.3	1	Imatinib intolerant
03	F/50	6/28/05	Chronic phase	44.2	1	Imatinib intolerant stopped 2 months prior
04	F/45	5/30/06	Chronic phase	35.6	1	Nilotinib
05	F/73	7/19/07	Chronic phase	12.5	0	Imatinib 300 mg daily
06	F/34	9/25/07	Chronic phase	350	1	None
07	F/51	10/8/07	Chronic phase	14.4	0	Dasatinib + hydroxyurea
08^	M/67	10/8/07	Chronic phase	23.8	0	None
09^	M/48	10/9/07	Chronic phase	23.6	0	None
10	F/24	10/20/07	Chronic phase	430	1	None
11	M/52	3/07/06	Accelerated phase	3.3	2	MK-0457 (VX-680)
12	F/80	12/15/00	Lymphoid blast crisis	46.2	93	Imatinib 300 mg daily
13	M/59	1/25/01	Myeloid blast crisis	14.7	87	Hydroxyurea
14	F/32	3/4/02	Myeloid blast crisis	20.1	82	Imatinib stopped 2 weeks prior
15	M/50	1/3/03	Myeloid (M7) blast crisis [a]	4.4	80	Imatinib 600 mg daily
16*	F/66	12/1/04	Myeloid blast crisis	9.3	43	Imatinib 300 mg daily stopped 1 wk before
17a*^	M/31	3/16/06	Myeloid blast crisis	40.1	79	Hydroxyurea
b*^		4/18/06	Myeloid blast crisis	3.5	55	Nilotinib
c*^		5/23/06	Myeloid blast crisis	55.3	80	Nilotinib + hydroxyurea
18*^	M/59	3/24/06	Myeloid blast crisis	51.5	74	Dasatinib stopped 2 weeks before
19	M/81	5/30/06	Myeloid blast crisis	6.0	77	Imatinib 600 mg daily
20a*	F/57	6/26/06	Myeloid blast crisis	0.9	5	Dasatinib
b*		8/10/06	Myeloid blast crisis	7.9	32	None
21	M/37	4/27/07	Myeloid blast crisis	42.0	83	Hydroxyurea
22	M/82	11/18/07	Myeloid blast crisis[b]	28.9	32	None
23^	M/49	5/20/08	Myeloid blast crisis	31.1	50	None
24	M/49	4/26/08	Myeloid blast crisis	43.6	2	Dasatinib
25	M/	1995	Myeloid blast crisis	N/A	N/A	none

[a] M7: Megakaryocytic blast crisis per the French-American-British (FAB) classification.
[b] P190 BCR-ABL positive CML, chronic myeloid leukemia; WBC, white blood cell; PB, peripheral blood.
*m-GSK3b positive by sequencing.
^m-GSK3b positive by PCR.

Table S2*A*. GSK3β, APC, Axin 1, β-catenin, c-myc, Cyclin D1 and LEF-1 Genomic and cDNA Sequencing Analysis: Normal Cord Blood (CB; $n = 3$), Peripheral Blood (PB; $n = 10$) and Bone Marrow (BM; $n = 2$) $N = 15$ samples, $n = 49$ subpopulations

Sample number	Sample	Subpopulation	gDNA	cDNA	% Isoforms
1	Normal CB1	HSC	NVD	NA	
1	Normal CB1	HSC	NVD	NA	
1	Normal CB1	HSC	NVD	NA	
1	Normal CB1	HSC	NVD	NA	
1	Normal CB1	GMP	NVD	NA	
1	Normal CB1	GMP	NVD	NA	
1	Normal CB1	GMP	NVD	NA	
1	Normal CB1	GMP	NVD	NA	
2	Normal CB2	HSC	NVD	NA	
2	Normal CB2	HSC	NVD	NA	
2	Normal CB2	HSC	NVD	NA	
2	Normal CB2	HSC	NVD	NA	
3	Normal CB3	HSC	NA	GSK3β exon 9 del	50
4	Normal PB1	HSC	NVD	NA	
4	Normal PB1	34^+38^+	NVD	NA	
4	Normal PB1	GMP	NVD	NA	
4	Normal PB1	Lin^+	NVD	NA	
5	Normal PB2	HSC	NVD	NA	
5	Normal PB2	34^+38^+	NVD	NA	
5	Normal PB2	GMP	NVD	NA	
6	Normal PB3	34^+38^-	NA	GSK3β exon 9 del	100
6	Normal PB3	34^+38^+	NA	GSK3β exon 9 del	50
6	Normal PB3	GMP	NA	GSK3β exon 9 del	100
6	Normal PB3	Lin^+	NA	GSK3β exon 9 del	30
7	Normal PB4	34^+38^+	NA	GSK3β exon 9 del	100
7	Normal PB4	Lin^+	NA	GSK3β exon 9 del	30
8	Normal PB5	34^+38^-	NA	GSK3β exon 9 del	50–100
8	Normal PB5	CMP	NA	GSK3β exon 9 del	50–100
8	Normal PB5	GMP	NA	GSK3β exon 9 del	100
8	Normal PB5	MEP	NA	GSK3β exon 9 del	50
8	Normal PB5	34^+38^-	NA	GSK3β exon 9 del	100
8	Normal PB5	34^+38^+	NA	GSK3β exon 9 del	100
8	Normal PB5	Lin^+	NA	GSK3β exon 9 del	30
9	Normal PB6	34 + 38-	NA	GSK3β exon 9 del	100
9	Normal PB6	34 + 38+	NA	GSK3β exon 9 del	50
9	Normal PB6	GMP	NA	GSK3β exon 9 del	100
9	Normal PB6	Lin+	NA	GSK3β exon 9 del	30
10	Normal PB7	34 + 38+	NA	GSK3β exon 9 del	100
10	Normal PB7	Lin+	NA	GSK3β exon 9 del	30
11	Normal PB8	CD34-enriched	NA	GSK3β exon 9 del	20
11	Normal PB8	CD34-negative	NA	GSK3β exon 9 del	20
12	Normal PB9	CD34-enriched	NA	GSK3β exon 9 del	20
12	Normal PB9	CD34-negative	NA	GSK3β exon 9 del	20
13	Normal PB10	CD34-enriched	NA	GSK3β exon 9 del	20
13	Normal PB10	CD34-negative	NA	GSK3β exon 9 del	20
14	Normal BM1	HSC	NA	GSK3β exon 9 del	50
14	Normal BM1	GMP	NA	GSK3β exon 9 del	50
15	Normal BM2	HSC	NA	APC E1317Q	50
15	Normal BM2	GMP	NA	APC E1317Q	50

NVD, no variant detected; NA, not analyzed; Del, deletion; HSC, hematopoietic stem cell; Lin^+, lineage-positive; gDNA, genomic DNA.

Table S2*B*. GSK3β, APC, Axin 1, β-catenin, c-myc, Cyclin D1 and LEF-1 Genomic and cDNA Sequencing Analysis: Chronic Phase (CP) CML (n = 4), Accelerated phase (AP) CML (n = 1), Myeloid Blast Crisis (BC) CML (n = 8), Lymphoid BC CML (n = 1); N = 18 samples, N = 51 subpopulations; Peripheral Blood (PB) and Bone Marrow (BM)

Patient number	Sample	Subpopulation	gDNA	cDNA	% transcripts
1	CP CML PB	34^+38^-	NA	GSK3β exon 9 del	100
1	CP CML PB	34^+38^+	NA	GSK3β exon 9 del	100
1	CP CML PB	Lin^+	NA	GSK3β exon 9 + 11 del	100
2	CP CML PB	34^+38^-	NA	GSK3β exon 8 + 9 del	100
2	CP CML PB	34^+38^+	NA	GSK3β exon 8 + 9 del	100
2	CP CML PB	Lin^+	NA	NA	
3	CP CML PB	34^+38^-	NA	GSK3β exon 9 del	100
3	CP CML PB	34^+38^-	NA	NA	
3	CP CML PB	Lin^+	NA	GSK3β exon 9 del	100
4	CP CML PB	HSC	NVD	NA	
4	CP CML PB	34^+38^+	NVD	NA	
4	CP CML PB	Lin^+	NVD	NA	
11	AP CML BM	HSC	NVD	NA	
11	AP CML BM	HSC	NVD	NA	
11	AP CML BM	HSC	NVD	NA	
11	AP CML BM	HSC	NVD	NA	
11	AP CML BM	GMP	NVD	NA	
11	AP CML BM	GMP	NVD	NA	
11	AP CML BM	GMP	NVD	NA	
11	AP CML BM	GMP	NVD	NA	
12	lymphoid BC CML BM	HSC	NA	NVD	
12	lymphoid BC CML BM	Lin^+	NA	NVD	
13	myeloid BC CML BM	HSC	NA	GSK3β exon8 + 9 del	50
13	myeloid BC CML BM	GMP	NA	GSK3β exon8 + 9 del	100
13	myeloid BC CML BM	Lin^+	NA	NVD	
14	myeloid BC CML BM	GMP	NA	NVD	
14	myeloid BC CML BM	Lin^+	NA	NVD	
15	myeloid BC CML BM	HSC	NA	NVD	
15	myeloid BC CML BM	GMP	NA	NVD	
17a	myeloid BC CML BM	HSC	NVD	GSK3β exon8 + 9 del	50
17a	myeloid BC CML PB	GMP	NVD	GSK3β exon8 + 9 del	100
17a	myeloid BC CML PB	Lin^+	NVD	GSK3β exon 9 del	100
17b	myeloid BC CML BM	HSC	NA	NA	
c-myc AAC > AGC N11S dbSNP: rs4645959					
17b	myeloid BC CML BM	GMP	NA	GSK3β exon 8 + 9 del	100
17b	myeloid BC CML BM	Lin^+	NA	GSK3β exon 11 del	50
17c	myeloid BC CML PB	HSC	NVD	GSK3β exon 9 del	50
17c	myeloid BC CML PB	GMP	NVD	GSK3β exon8 + 9 del	100
17c	myeloid BC CML PB	Lin^+	NVD	GSK3β exon 9 del	100
18	myeloid BC CML BM	HSC	NVD	GSK3β exon 9 del	60
18	myeloid BC CML BM	HSC	NA	c-myc TTT > TAT F375Y	50
18	myeloid BC CML BM	GMP	NA	GSK3β exon 8 + 9 del	100
18	myeloid BC CML BM	Lin^+	NA	GSK3β exon 9 del	50
19	myeloid BC CML BM	HSC	NVD	NA	
19	myeloid BC CML BM	GMP	NVD	NA	
19	myeloid BC CML BM	Lin^+	NVD	NA	
20	myeloid BC CML PB	HSC	NVD	GSK3β exon8 + 9 del	50
20	myeloid BC CML PB	$CD34^+CD38^+$	NVD	GSK3β exon8 + 9 del	50
20	myeloid BC CML PB	Lin^+	NVD	GSK3β exon 9 del	100

NVD, no variant detected; NA, not analyzed; Del, deletion; HSC, hematopoietic stem cell; GMP, granulocyte-macrophage progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythroid progenitor; Lin^+, lineage-positive.